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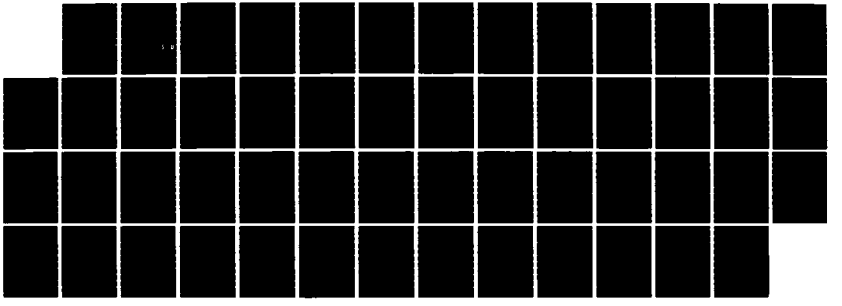

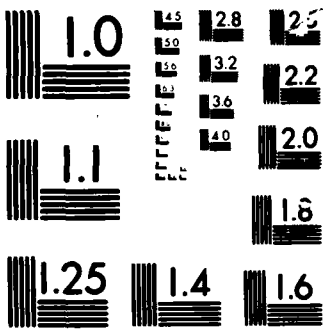

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AD-A168 500

IN VIVO SCREENING OF RADIOPROTECTORS

ANNUAL SUMMARY REPORT

Jacob J. Clement, Ph.D.

June, 1983

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

During the current contract year, 28 agents were evaluated for drug toxicity and 30 agents were tested for radioprotectant activity. A total of 35 new agents were evaluated in all. Eleven of the compounds tested protected irradiated mice and produced 70% survival or greater in mice exposed to a radiation dose lethal to nonprotected animals. Only one agent, a relatively nontoxic phosphorothioate (WR 250397), protected 100% of treated mice at a dose of 1200 mg/kg. The radioprotectant standard WR 2721, for comparison, provides 100% protection when administered at doses between 200 and 600 mg/kg.

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Six of eight agents identified by the COTR as being of particular interest to WRAIR were entered into detailed evaluation for radioprotectant activity. Detailed testing includes primary protectant screening and determination of: degree of protection, time of maximum protection, duration of protection, and effectiveness of oral drug formulation. Radiation dose modification studies conducted with intraperitoneally administered WR 2721 and WR 168643 indicated a Dose Modification Factor (DMF) of 2.2 and 1.1 for WR 2721 and WR 168643, respectively. WR 168643 and WR 3689 given orally produced DMF values of 1.3 and 1.5, respectively. Duration of protection with orally administered WR 2721 exceeded 90 minutes. Radiation protection with orally or intraperitoneally administered WR 168643 lasted approximately 30 minutes. Protection with WR 3689 appeared to last less than one hour following ip injection but was greater than two hours following oral injection. Preliminary toxicity and radioprotection screening studies were completed for three other known radioprotectant compounds WR 1065, WR 77913, and WR 176542.

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In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

SUMMARY

This report describes results of in vivo screening of potential radiation protectant agents as part of the USAMRDC antiradiation drug development program. During this contract year, drug toxicity determinations were completed for 28 agents and preliminary radioprotectant testing was performed for 30 agents. Eleven of the compounds tested protected 70% or greater of treated groups of mice receiving a lethal dose of radiation. Only one agent, a relatively nontoxic phosphorothioate (WR 250397), protected 100% of treated mice at a dose of 1200 mg/kg. For comparison the radioprotectant standard WR 2721 provides 100% protection when administered at doses between 200 and 600 mg/kg.

Six agents of eight identified by the COTR as being of particular interest are undergoing detailed evaluation. Testing of these agents will consist of preliminary toxicity and radioprotectant screening followed by formal determination of drug toxicity (LD_{50}), time of maximum radioprotection, duration of protection, degree of protection (DMF), and protection of both hematopoietic and gastrointestinal tissues. These agents are given orally or parenterally. Radiation dose modification studies were conducted for WR 2721 ip administered and WR 168643, DMF values of 2.2 and 1.1 were determined. For drugs given orally, WR 168643 and WR 3689 produced DMF values of 1.3 and 1.5, respectively. Duration of protection with orally administered WR 2721 exceeded 90 minutes while protection with WR 168643 lasted approximately 30 minutes following oral or ip injection. Protection with WR 3689 appeared to last less than one hour following ip injection but was greater than two hours following oral injection. Preliminary toxicity and radioprotection screening studies were also completed for the known radioprotectant compounds WR 1065, WR 77913, and WR 176542.

I. INTRODUCTION

Medical illness produced as a result of radiation exposure from nuclear weapons vary in the degree of severity and time of onset. Radiation doses of 2000 rads and above cause convulsions, tremors, ataxia, and loss of consciousness from minutes to hours after exposure. Moderate radiation doses between 500 and 1000 rads causes nausea, vomiting, and diarrhea which usually begins in three to five days. Exposure to relatively low doses of 200 to 300 rads results in fever, fatigue, infection, and hemorrhaging which appear two to three weeks after exposure. All three radiation syndromes result in mortality with survival possible only at doses below about 500 rads.

Protection of combat personnel from either prompt or fallout radiation from nuclear weapons can decrease casualties and increase the duration of personnel performance by decreasing both the onset and severity of radiation illness. One of the more promising methods of protection is a self-administrable drug which can protect individuals from radiation damage. Both the U. S. Air Force Radiation Laboratory and the U. S. Army Medical Research and Development Command (USAMRDC) have tested antiradiation drugs. The Air Force program has tested over 1500 compounds, and the USAMRDC program has tested over 4,400 compounds. Only about 200 of these compounds have shown activity in mice; and of these compounds, relatively few appear potentially useful in humans. To be of military use, an antiradiation drug should protect personnel within minutes after administration (preferably orally), be effective for several hours, have no serious side effects, and be suitable for multiple dosing over a relatively short period of time. The degree of protection is also important. While total radiation protection is desirable, a reduction by a factor of 2-3 in the effective radiation dose appears to be a reasonable expectation and an attainable goal. Towards this end, the USAMRDC sponsors a rational

drug development program based on established structure-activity relationships, as well as on promising new directions. The purpose of this contract, "In Vivo Screening of Radioprotectors," is to evaluate the effectiveness of newly synthesized chemoprophylatic agents and to characterize in detail promising agents which have shown protectant activity in an in vivo screen. Data presented in this report represent the results of our testing efforts during the period from March 1982 to April 1983. The studies described include: (a) drug toxicity of 28 new chemical agents; (b) radioprotection screening of 30 new chemical agents; and (c) additional testing of six known radioprotectants selected by WRAIR for detailed evaluation.

II. MATERIALS AND METHODS

A. EXPERIMENTAL ANIMALS

All animals used for experiments in this research effort were C57BL/6 female mice from Charles River Breeding Laboratories, Wilmington, Massachusetts supplied as having a positive Sendai titer and in Pseudomonas-free condition. Animals are delivered in filtered crates and housed five to a cage with each cage fitted with an individual filter cover. Animals are tested for Pseudomonas prior to use and infected mice are immediately disposed of. Each group of mice is handled with sterile forceps and clean gloves. During quarantine and after treatment, animals are kept on a 12-hour light cycle and allowed laboratory chow and hyperacidified water (pH 2.5) ad libitum. All experimental animals are 11-12 weeks of age. No anesthetic was used in these studies. Mice surviving 30 days after drug treatment or irradiation are euthanized in a CO_2 chamber and incinerated.

B. EXPERIMENTAL DRUGS

All compounds were supplied by the COTR or his designated representative. Samples are stored as indicated on the shipping list or bottle, refrigerated or frozen with drierite if hygroscopic. All compounds are shielded from light. Prior to use, samples are allowed to warm to room temperature. Agents are weighed under a chemical fume hood and formulated in appropriate vehicle immediately prior to injection. Injections are given at 1% of individual body weight. All drug doses used in this report are corrected for salt and water content.

C. TESTING PROCEDURES

Test animals are weighed by group in sterile, disposable containers on Days 0, 5, and 30 post-treatment. Acute drug toxicity was determined from animal lethality up to 30 days following drug administration using five mice per drug dose. Radioprotectant activity of test compounds was determined from group survival rates of mice at 30 days following treatment with the test drug and a dose of radiation previously demonstrated to be lethal to 100% of unprotected animals ($LD_{100/30}$). Animals are irradiated in clean disposable containers in groups of 10 mice on a rotating turntable. The irradiation unit is a J. L. Shepherd, Mark I, Model 68 ^{137}Cs-irradiation unit (Serial #647) which produces a dose rate of 285 rads/min. The positive reference control consists of WR 2721 (BK 02762) given intraperitoneally at 500 mg/kg 30 minutes prior to irradiation.

III. RESULTS AND DISCUSSION

Data presented in this report represent results of work accomplished since the submission of the second annual report (March 1982). Experiments were performed between March, 1982 and March, 1983. Work initiated and completed during this time period is listed in Table 1. These studies are broken down into the following sections:

- o Preliminary screening of newly synthesized compounds which involves acute toxicity testing or preliminary radioprotectant screening of a total of 35 potential radioprotectors.

- o Detailed evaluation of agents of interest to WRAIR which involves specific investigation of the activity of six known radioprotectors.

A. PRELIMINARY SCREENING OF NEWLY SYNTHESIZED COMPOUNDS

Thirty-five compounds were received and evaluated as potential radioprotectors during the past year. Twenty-eight agents were tested for acute toxicity and 30 agents were evaluated in the radioprotector screen. The compounds were synthesized and submitted by nine laboratories; however, the majority of compounds came from three investigators as shown in Table 2.

Of the 28 compounds tested for toxicity as described in Table 3, six were quite toxic (toxic at less than 40 mg/kg) and seven were relatively nontoxic (no lethality at 600 mg/kg). Some of the compounds presented problems in formulation. WR 250292 (BK 39465), for example, was a gummy solid which proved quite difficult to retrieve from the glass ampoule in which it was stored. Two of the compounds, WR 250281

Table 1

STUDIES INCLUDED IN THIS REPORT

Study	Drug Tested, WR (BN)	Test
WR 33	249939 (BJ 13194)	Acute toxicity
	250021 (BJ 16211)	Acute toxicity
	250022 (BJ 16220)	Acute toxicity
	250023 (BJ 16202)	Acute toxicity
WR 34	249939 (BJ 13194)	Acute toxicity
	250023 (BJ 16202)	Acute toxicity
WR 35	250022 (BJ 16220)	Radioprotector screen
	250021 (BJ 16211)	Radioprotector screen
WR 36	249939 (BJ 13194)	Radioprotector screen
	250023 (BJ 16202)	Radioprotector screen
	3689 (BJ 78538)	Radioprotection time course
WR 37	249599 (BJ 90945)	Radioprotector screen
	249600 (BJ 90936)	Radioprotector screen
	248778 (BJ 63377)	Radioprotector screen
	249706 (BK 01827)	Radioprotector screen
WR 38	3689 (BJ 78538)	Radiation dose modification
	2721 (BK 02762)	Radiation dose modification
WR 39	250083 (BK 20653)	Acute toxicity
	250084 (BK 20662)	Acute toxicity
WR 40	250083 (BK 20653)	Radioprotector screen
	250084 (BK 20662)	Radioprotector screen
	248836 (BJ 64007)	Radioprotector screen
WR 41	168643 (BJ 44774)	Radiation dose modification
WR 42	168643 (BJ 44774)	Radiation dose modification
	249716 (BK 02164)	Radioprotector screen
WR 43	77913 (BJ 78529)	Radioprotector screen
	176542 (BJ 44569)	Radioprotector screen
WR 44	2721 (BK 02762)	Duration of protection annual characterization of rad. resp. C57BL/6 female

Table 1

(contd)

Study	Drug Tested, WR (BN)	Test
WR 45	135023 (BK 24080)	Acute toxicity
	2721 (BK 02762)	Acute toxicity
WR 46	250121 (BK 22200)	Radioprotector screen
	135023 (BK 24080)	Radioprotector screen
WR 47	250281 (BK 38182)	Acute toxicity
	250282 (BK 38173)	Acute toxicity
	250293 (BK 39474)	Acute toxicity
	250292 (BK 39465)	Acute toxicity
	091283 (BK 39456)	Acute toxicity
WR 48	250281 (BK 38182)	Acute toxicity
	250293 (BK 39474)	Acute toxicity
WR 49	091283 (BK 39456)	Radioprotector screen
	250281 (BK 38182)	Radioprotector screen
	250282 (BK 38173)	Radioprotector screen
WR 50	250292 (BK 39465)	Radioprotector screen
	250293 (BK 39474)	Radioprotector screen
WR 51	250288 (BK 39330)	Acute toxicity
	250345 (BK 45605)	Acute toxicity
WR 52	250288 (BK 39330)	Radioprotector screen
	250345 (BK 45605)	Radioprotector screen
WR 53	250346 (BK 45614)	Acute toxicity
	249262 (BJ 78618)	Acute toxicity
WR 54	250346 (BK 45614)	Radioprotector screen
	249262 (BJ 78618)	Radioprotector screen
	250292 (BK 39465)	Radioprotector screen
	250293 (BK 39474)	Radioprotector screen
WR 55	247119 (BJ 91531)	Acute toxicity
	250393 (BK 46899)	Acute toxicity
	250394 (BK 46906)	Acute toxicity
	169402 (BK 46942)	Acute toxicity
	250397 (BK 46933)	Acute toxicity
	250455 (BK 48606)	Acute toxicity
	250392 (BK 46871)	Acute toxicity

Table 1

(contd)

Study	Drug Tested, WR (BN)	Test
WR 56	1065 (BJ 05030)	Acute toxicity
	250456 (BK 48615)	Acute toxicity
	250393 (BK 46899)	Acute toxicity
	169402 (BK 46942)	Acute toxicity
	250397 (BK 46933)	Acute toxicity
	250455 (BK 48606)	Acute toxicity
WR 57	247119 (BJ 91531)	Radioprotector screen
WR 58	169402 (BK 46942)	Radioprotector screen
WR 58B	250393 (BK 46899)	Radioprotector screen
	250397 (BK 46933)	Radioprotector screen
WR 59	250394 (BK 46906)	Radioprotector screen
	1065 (BJ 05030)	Radioprotector screen
WR 60	250392 (BK 46871)	Radioprotector screen
	250455 (BK 46806)	Radioprotector screen
	250456 (BK 48615)	Radioprotector screen
WR 61	2721 (BK 02762)	Radioprotection vs. drug dose
WR 62	28210 (BK 48599)	Acute toxicity
	250368 (BK 46264)	Acute toxicity
	250525 (BK 50642)	Acute toxicity
	250524 (BK 50633)	Acute toxicity

Table 2

NEWLY SYNTHESIZED COMPOUNDS EVALUATED

Submitter	Toxicity	Radioprotection
L. Field Vanderbilt University	8	11
L. Bauer University of Illinois Med. Center	10	10
J. Piper Southern Research Institute	4	3
Other Friedheim, Rockefeller University (1) Ash Stevens, Inc. (1) G. H. Lord, Johnson & Johnson (1) Southwest Research Institute (2) E. Bueding, Johns Hopkins University (1) D. Pearson, Vanderbilt University (1)	6	6
Total	28	30

Table 3

SUMMARY OF ACUTE TOXICITY OF NEWLY SYNTHESIZED COMPOUNDS

Agent		Vehicle	Dose (mg/kg)*	Route	Lethality/ Total	30-Day Survivors (%)
WR	BN					
28210	BK 48599	10% EtOH + 90% H_2O	600	ip	0/5	100
			300		0/5	100
			150		0/5	100
			75		0/5	100
91823	BK 39456	20% EtOH + 80% Klucel	600	ip	5/5	0
			300		0/5	100
			150		0/5	100
			75		0/5	100
135023	BK 24080	H_2O	600	ip	5/5	0
			300		0/5	100
			150		0/5	100
			75		0/5	100
			37.5		0/5	100
			18.75		0/5	100
169402	BK 46942	Klucel	600	ip	5/5	0
			300		5/5	0
			150		5/5	0
			75		5/5	0
			37.5		3/5	40
247119	BJ 91531	H_2O	600	ip	4/5	20
			300		3/5	40
			150		0/5	100
			75		0/5	100
249262	BJ 78618	H_2O	600	ip	5/5	0
			300		1/5	80
			150		0/5	100
			75		0/5	100
249939	BK 13194	20% EtOH + TW 80	600	ip	5/5	0
			300		5/5	0
			150		5/5	0
			75		5/5	0
			37.5		5/5	0
			18.75		3/5	40
			9.38		0/5	100
250021	BK 16211	20% EtOH + TW 80	600	ip	5/5	0
			300		5/5	0
			150		5/5	0
			75		5/5	0

Table 3

(contd)

Agent WR	Agent BN	Vehicle	Dose (mg/kg)*	Route	Lethality/ Total	30-Day Survivors (%)
250022	BK 16220	20% EtOH + TW 80	600	ip	5/5	0
			300		5/5	0
			150		5/5	0
			75		3/5	40
250023	BK 16202	10% EtOH + TW 80	600	ip	5/5	0
			300		5/5	0
			150		5/5	0
			75		5/5	0
			37.5		5/5	0
			18.75		3/5	40
			9.38		0/5	100
250083	BK 20653	20% EtOH + 80% H_2O	300	ip	5/5	0
			150		5/5	0
			75		5/5	0
			37.5		1/5	80
			18.8		0/5	100
250084	BK 20662	20% EtOH + 80% H_2O	300	ip	4/5	20
			150		5/5	0
			75		4/5	20
			37.5		1/5	80
			18.8		0/5	100
250281	BK 38182	20% DMSO + 80% H_2O	120	ip	3/5	40
			60		1/5	80
			30		0/5	100
			15		0/5	100
		Klucel	120	ip	1/5	80
			60		0/5	100
			30		0/5	100
250282	BK 38173	20% EtOH + 20% Emulphor + 60% Saline	102	ip	5/5	0
			51		0/5	100
			25.5		0/5	100
			12.75		0/5	100
250288	BK 39330	H_2O	600	ip	5/5	0
			300		2/5	60
			150		0/5	100
			75		0/5	100
250292	BK 39465	10% DMSO + 90% H_2O	600	ip	0/5	100
			300		0/5	100
			150		0/5	100
			75		0/5	100

Table 3

(contd)

Agent WR	Agent BN	Vehicle	Dose (mg/kg)*	Route	Lethality/ Total	30-Day Survivors (%)
250293	BK 39474	H_2O	600	ip	5/5	0
			300		5/5	0
			150		0/5	100
			75		0/5	100
		Klucel	300	ip	5/5	0
			150		1/5	80
			75		0/5	100
250345	BK 45605	Klucel	600	ip	0/5	100
			300		0/5	100
			150		0/5	100
			75		0/5	100
250346	BK 45614	Peanut Oil	600	ip	5/5	0
			300		5/5	0
			150		1/5	80
			75		0/5	100
250368	BK 46264	H_2O	600	ip	5/5	0
			300		5/5	0
			150		4/5	20
			75		0/5	100
250392	BK 46871	H_2O	600	ip	1/5	80
			300		0/5	100
			150		0/5	100
			75		0/5	100
250393	BK 46899	20% EtOH + 80% H_2O	600	ip	5/5	0
			300		5/5	0
			150		5/5	0
			75		5/5	0
			37.5		5/5	0
250394	BK 46906	20% EtOH + 80% H_2O	600	ip	5/5	0
			300		5/5	0
			150		5/5	0
			75		0/5	100
250397	BK 46933	H_2O	1200	ip	1/5	80
			600		0/5	100
			300		0/5	100
			150		0/5	100
			75		0/5	100

Table 3

(contd)

Agent WR	Agent BN	Vehicle	Dose (mg/kg)*	Route	Lethality/ Total	30-Day Survivors (%)
250455	BK 48606	H_2O	600	ip	5/5	0
			300		5/5	0
			150		5/5	0
			75		5/5	0
			37.5		0/5	100
250456	BK 48615	H_2O	600	ip	0/5	100
			300		0/5	100
			150		0/5	100
250524	BK 50633	H_2O	600	ip	0/5	100
			300		0/5	100
			150		0/5	100
			75		0/5	100
250525	BK 50642	H_2O	600	ip	0/5	100
			300		0/5	100
			150		0/5	100
			75		0/5	100

*Corrected for salt and water content

(BK 38182) and WR 250293 (BK 39474) were tested in alternate vehicles to determine if the original vehicle modified toxic dose levels.

The results of the 30 agents evaluated as radioprotectors are summarized in Table 4. The data for only one indicates complete radioprotection. The data for 10 other compounds indicates moderate protection of more than 70% and eight of the agents protected only slightly (protection of more than 40%).

The compounds tested fall into four groups according to the submitter to the WRAIR testing program. The first group consists of 10 amidinium compounds synthesized in the laboratory of Dr. Ludwig Bauer of the University of Illinois (Table 5). All of the compounds of this group provided some degree of radioprotection; however, the data suggest no agent as active as the reference compound WR 2721. The monomeric totyl-adamantane (WR 249939) provided 80% protection at the highest nontoxic dose of 8.0 mg/kg. WR 250021, WR 250022, and WR 250023 are all related methoxy phenyl adamantyl amidiniums. WR 250023 provided 50% protection; the phosphorthioate-substituted agent (WR 250022) provided 60% protection; and WR 250021, the disulfide dihydrochloride dimer, provided 90% protection to treated animals at a maximum dose of 15 mg/kg. This was the maximum protection of any compound in the group of adamantyl amidiniums. WR 250083 is a fluorophenyl adamantyl amidinium phosphorothioate. WR 250084 is a disulfide dimer of that compound. Both compounds protected a maximum 80% of the animals at a dose which produced no drug related lethalities. WR 250281 and WR 250282 are methyl thiophenyl amidiniums. The former is an ethyl thiosulfate-substituted compound which provided a maximum of 60% protection. The latter is a phosphorthioate-substituted compound which protected 70% of treated animals. WR 250393 protected 60% of the treated animals at 20 mg/kg. This agent is a thienyl amidinium chloride. WR 250394 is a benzyl amidinium disulfide dihydrochloride dimer which was the least toxic of all the amidiniums but protected only 60% of irradiated animals.

Table 4

SUMMARY OF RADIOPROTECTANT ACTIVITY OF NEWLY SYNTHESIZED COMPOUNDS

Agent WR	Agent BN	Vehicle	Route	Time Before Rad (min)	Dose (mg/kg)[a]	Lethality/ Total	30-Day Survivors (%)[b]
091283	BK 39456	20% EtOH + 80% Klucel	ip	15	400	7/10	20
					200	0/10	70
					100	0/10	60
135023	BK 24080	H_2O	ip	30	500	5/10	0
					250	0/10	0
					125	0/10	0
					62.5	0/10	0
169402	BK 46942	Klucel	ip	30	32	5/10	0
					16	0/10	0
					8	0/10	0
247119	BJ 91531	H_2O	ip	30	200	10/10	0
					100	0/10	0
					50	0/10	0
248778	BJ 63377	Klucel	ip	15	600	1/6	0
					300	0/10	20
					150	0/10	0
248836	BJ 64007	H_2O	ip	15	134	0/10	90
					66.8	0/10	30
					37.5	0/10	0
					18.8	0/10	0
249262	BJ 78618	H_2O	ip	30	300	10/10	0
					150	1/10	70
					75	0/10	10
249706	BK 01827	H_2O	ip	15	91.5	3/10	10
					45.8	0/10	0
					22.9	0/10	0
					11.4	0/10	0
249716	BK 02164	H_2O	ip	15	600	0/10	0
					300	0/10	0
					150	0/10	0
					75	0/10	0
249599	BJ 90945	Klucel	ip	15	800	10/10	0
					400	1/10	30
					200	0/10	40
					100	0/10	20
249600	BJ 90936	Klucel	po	30	600	2/10	50
					300	0/10	40
					150	0/10	20

Table 4

(contd)

Agent WR	Agent BN	Vehicle	Route	Time Before Rad (min)	Dose (mg/kg)[a]	Lethality/ Total	30-Day Survivors (%)[b]
249939	BK 13194	20% EtOH + 80% H_2O + TW 80	ip	30	16	6/10	30
					8	0/10	80
					4	0/10	10
					2	0/10	30
250021	BK 16211	20% EtOH + 80% H_2O + TW 80	ip	30	60	8/10	10
					30	10/10	0
					15	0/10	90
					7.5	0/10	20
250022	BK 16220	20% EtOH + 80% H_2O + TW 80	ip	30	60	7/10	20
					30	0/10	60
					15	0/10	30
					7.5	0/10	0
250023	BK 16202	10% EtOH + 90% H_2O + TW 80	ip	30	16	0/10	50
					8	0/10	30
					4	0/10	0
					2	0/10	0
250083	BK 20653	20% EtOH + 80% H_2O	ip	30	36	6/10	40
					18	0/10	60
					9	0/10	80
250084	BK 20662	20% EtOH + 80% H_2O	ip	30	36	10/10	0
					18	8/10	20
					9	0/10	80
250121	BK 22200	Special	po	Day 4[c]	1000	0/10	0
				Day 4	500	0/10	0
				Day 0, 30	1000	0/10	0
				Day 0, 30	500	0/10	0
250281	BK 38182	Klucel	ip	30	60	1/10	50
					30	0/10	60
					15	0/10	50
250282	BK 38173	20% EtOH + 20% Emulphor + 60% Saline	ip	30	60	1/10	70
					30	0/10	70
					15	0/10	30
250288	BK 39330	H_2O	ip	30	300	5/10	40
					150	0/10	30
					75	0/10	20
250292	BK 39465	10% DMSO + 90% H_2O	ip	15	1000	10/10	0
					500	4/10	0
					250	0/10	0

Table 4

(contd)

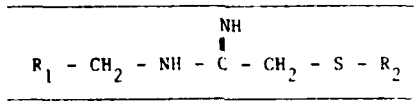
Agent WR	Agent BN	Vehicle	Route	Time Before Rad (min)	Dose (mg/kg)[a]	Lethality/ Total	30-Day Survivors (%)[b]
250293	BK 39474	Klucel	ip	15	150	4/10	60
					75	0/10	70
					37.5	0/10	10
250345	BK 45605	Klucel	ip	15	1200	5/10	50
					600	0/10	80
					300	0/10	80
250346	BK 45614	Peanut Oil	ip	15	150	1/10	0
					75	0/10	20
					37.5	0/10	0
250392	BK 46871	H_2O	ip	30	400	0/10	0
					200	0/10	0
					100	0/10	0
250393	BK 46899	20% EtOH + 80% H_2O	ip	30	20	0/10	60
					10	0/10	60
					5	0/10	0
250394	BK 46906	20% EtOH + 80% H_2O	ip	30	80	1/10	60
					40	0/10	0
					20	0/10	20
250397	BK 46933	H_2O	ip	30	1200	0/10	100
					600	0/10	70
					300	0/10	10
250455	BK 48606	H_2O	ip	15	60	0/10	0
					30	0/10	0
					15	0/10	0
250456	BK 48615	H_2O	ip	30	1200	0/10	0
					600	0/10	0
					300	0/10	0

[a] Corrected for salt and water content.
[b] Percent of mice surviving at 30 days after treatment with drug and whole-body irradiation of 1000 rads ($LD_{100/30}$).
[c] Schedule: drug: Day 0, Day 2; radiation: Day 4 or Day 0; drug 30 min before radiation.

Table 5

RADIOPROTECTION WITH AMIDINIUM COMPOUNDS:

L. BAUER, UNIVERSITY OF ILLINOIS MEDICAL CENTER



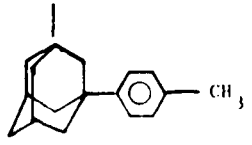
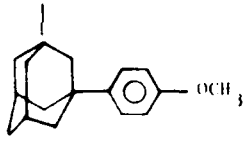
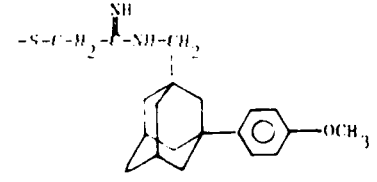
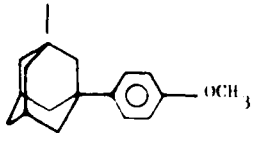
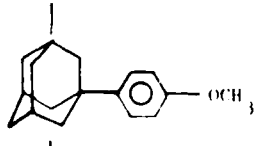
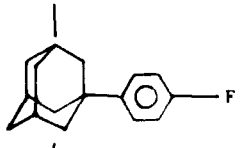
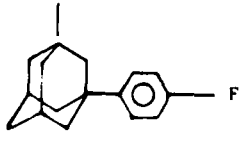
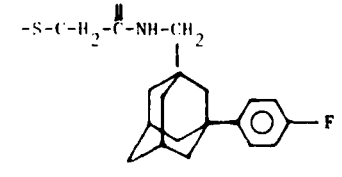
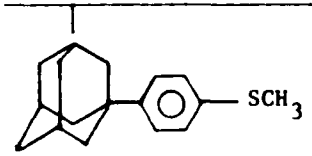
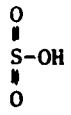
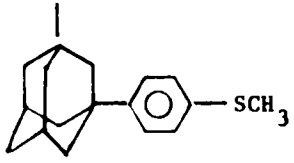
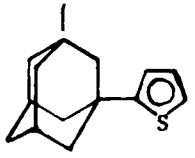
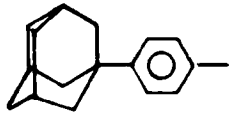
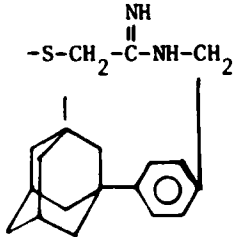
WR	BN	Structure R_1	Structure R_2	Maximum Protection (30-Day Survivors)	Dose (mg/kg)*
249939	BK 13194		$-SO_3H$	80%	8
250021	BK 16211			90%	15
250022	BK 16220		$-PO_3Na$	60%	30
250023	BK 16202		$-H$	50%	16
250083	BK 20653		$-PO_3Na$	80%	9
250084	BK 20662			80%	9

Table 5

(contd)

WR	BN	Structure		Maximum Protection (30-Day Survivors)	Dose (mg/kg)*
		R_1	R_2		
250281	BK 38182			60%	30
250282	BK 38173		$-PO_3Na$	70%	60,30
250393	BK 39474		-H	60%	20
250394	BK 46906			60%	80

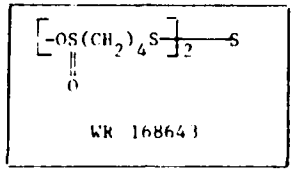
*Corrected for salt and water content

The agents synthesized in the laboratory of Dr. Lemar Field at Vanderbilt University (Table 6) comprise the largest group of radioprotectors screened. These analogs of WR 168643 range in activity from no protection (four compounds) to a maximum of 90% effectiveness for WR 248836 at the highest dose tested. This pentasulfide-substituted analog of WR 168643 had shown some activity in earlier testing and retesting was indicated because of Pseudomonas contamination. WR 249716 is a trisulfide analog of WR 168643 which was nontoxic at 600 mg/kg but provided no protection to treated animals. Three agents which were retested during this year were WR 249599, WR 249600, and WR 248778. The first is a pyrrolidinyldithiobutane-sulfonate which showed marginal protection. The other two WR 168643 analogs also provided only marginal protection at doses producing no drug related deaths. WR 250292, which is identified as 1,8-dioxa-4,5,9-triathia-12-cyclododecanone-9-oxide, provided no protection at any dose tested even at those doses which were partially toxic. Three dithiobutane sulfinates were tested - WR 250293, WR 250456, and WR 250345. The carboxyethyl-substituted compound (WR 250293) protected 70% of irradiated animals. The butylene-substituted compound (WR 250345) protected 80% of treated animals at the highest nontoxic dose tested and WR 250456 (the adamantyl-substituted compound) provided no protection at any dose up to 1200 mg/kg. WR 91283 is a carboxyethyl-containing thiosulfopropionic acid which protected 70% of irradiated animals. The dimethyl-substituted sulfinopropionate, WR 250346, and the disodium-substituted sulfinopropanoate, WR 250455, provided no significant protection at any dose tested.

Three radioprotectant agents were submitted by J. R. Piper of Southern Research Institute (Table 7). WR 249706 is an oxazaphosphorine which displayed no significant protective ability at nontoxic dosages tested. Two of the compounds are phosphorthioates related to WR 2721. The adamantyl-containing derivative, WR 169402, was not active at the highest tolerated dose of 16 mg/kg. The extremely nontoxic carbonyl-containing derivative, WR 250397, however, was protective to 100% of the irradiated animals at 1200 mg/kg.

Table 6

RADIOPROTECTION WITH VANDERBILT COMPOUNDS: L. FIELD



WR 168643

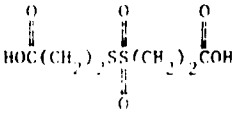
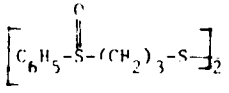
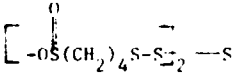
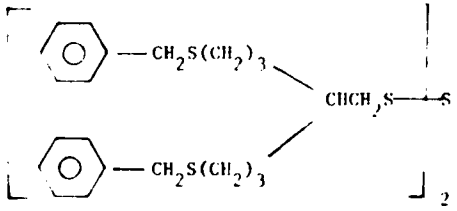
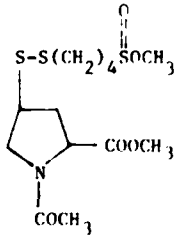
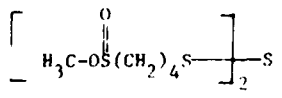
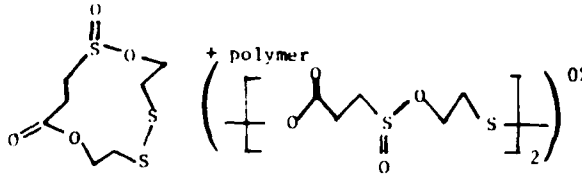
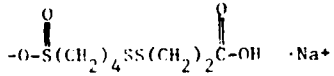
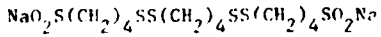
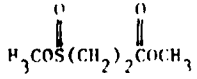
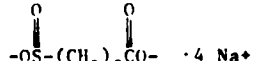
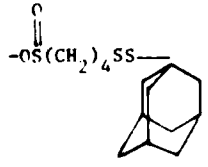
Agent WR	Agent BN	Structure	Maximum Protection (30-Day Survivors)	Dose (mg/kg) *
91283	BK 39456		70%	200
248778	BJ 63377		20%	300
248836	BJ 64007		90%	134
249716	BK 02164		0%	600
249599	BJ 90945		40%	200

Table 6

(contd)

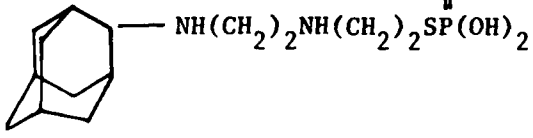
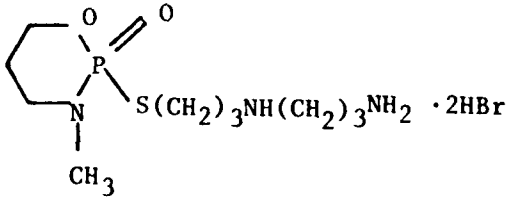
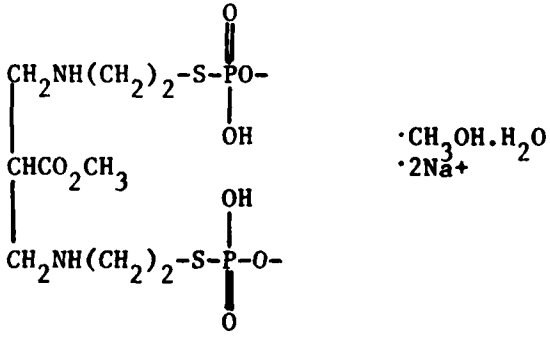
Agent WR	Agent BN	Structure	Maximum Protection (30-Day Survivors)	Dose (mg/kg)*
249600	BJ 90936		50%	600
250292	BK 39465		0%	250
250293	BK 39474		70%	75
250345	BK 45605		80%	600
250346	BK 46614		20%	75
250455	BK 48606		0%	60
250456	BK 48615		0%	1200

*Corrected for salt and water content

Table 7

RADIOPROTECTION WITH SOUTHERN RESEARCH INSTITUTE COMPOUNDS:

J. PIPER

Agent		Structure	Maximum Protection (30-Day Survivors)	Dose (mg/kg)
WR	BN			
169402	BK 46942		0%	16
249706	BK 01827		10%	91.5
250397	BK 46933		100%	1200

Six compounds tested as radioprotectors were unique compounds submitted from various sources (Table 8). Only two of the compounds displayed marginal activity. WR 249262 is a cobalt containing derivative of WR 2721 synthesized by Dr. Friedheim at Rockefeller University. The agent was synthesized as an antitrypanosomal drug with the presumed capacity to cross the blood-brain barrier. This would theoretically provide protection of the CNS from radiation damage. The compound protected 70% of the irradiated mice from hematopoietic injury. No assumptions can be made from these data relative to CNS protection. Another derivative of WR 2721, an arsenic-containing compound (WR 247119) which was synthesized by Ash Stevens, had no activity as a protector at any dose tested. WR 250288, a thiazolidinium-containing compound synthesized at Southwest Research Institute provided only 30% protection at the highest nontoxic dose of 150 mg/kg. 6-thio-fructose (WR 250392) was tested at the University of Pennsylvania with positive results in rats; however, no protection was provided in the radioprotector screen at the highest tolerated dose of 400 mg/kg. The compound was submitted by Dr. Geoffrey Lord of Johnson and Johnson. Don Pearson of Vanderbilt University submitted WR 135023. This "stable free-radical" was inactive at all doses tested. Oltipraz (WR 250121) is a compound submitted by Dr. Ernest Bueding of Johns Hopkins University. It was formulated as described by Dr. Bueding in the special vehicle containing 25% glycerol and 1% cremophor El and tested on two schedules. The first schedule claimed to be effective by Dr. Beuding was an oral drug administration on Days 0 and 2 followed by irradiation on Day 4. The second schedule was a single oral bolus injection followed by irradiation in 30 minutes. Data indicate that neither schedule was effective at either dose tested in protecting irradiated animals.

In summary, 37% of the compounds tested in the radioprotector screen provided moderate protection of more than 70%. The phosphorothioate WR 250397 was not effective over a wide dose range. Future testing of newly-synthesized agents will include confirmation of active radioprotectants. In addition to these studies acute toxicity

Table 8

RADIOPROTECTION WITH MISCELLANEOUS COMPOUNDS

Submitter	Compound Class	WR	BN	Maximum Protection (%)	Dose (mg/kg)
D. Pearson Vanderbilt University	Stable free-radical	135023	BK 24080	0	500
Ash Stevens	As-containing 2721 derivative	247119	BJ 91531	0	100
Freidheim Rockefeller University	Co-containing 2721 derivative	249262	BJ 78618	70	150
E. Bueding Johns Hopkins University	Oltipraz	250121	BK 22200	0	1000
Southwest Research	Thiazolidinium	250288	BK 39330	30	150
G. Lord Johnson & Johnson	6-Thio-fructose	250392	BK 46871	0	400

determinations will be scheduled for all submitted agents for which radioprotection studies were performed without preliminary toxicity data. These latter studies are instituted at the request of the interim COTR.

A summary of results for preliminary radioprotector screening during the period of performance of this contract is included as Table 9.

B. DETAILED EVALUATION

Detailed evaluation studies were begun for six agents during this contract year (Table 10). WR 2721, WR 168643, WR 3689, WR 77913, WR 176542, and WR 1065 are in various stages of the evaluation. For the first three known radioprotective agents, several detailed studies have been completed. For the latter three compounds, preliminary studies have been initiated in preparation for full evaluation.

WR 2721. The radioprotectant characteristics of WR 2721 are being determined to provide reference data for evaluating the relative efficacy of protectors of special interest to WRAIR. An analysis of the radiation dose modification properties of intraperitoneally administered WR 2721 was conducted at a drug dose of 500 mg/kg corresponding to the standard drug dose of WR 2721 used in screening studies. Results are shown in Table 11. The LD_{50} derived from these data was 1983 rads (95% CL = 1831-2149) with a probit slope of 10.73. The LD_{50} for unprotected irradiated mice is 809-907 rads using data reported in Report #4 and this report, respectively. Thus, the dose modification factor (DMF) lies between 2.5 and 2.2.

A duration of protection study was conducted using an LD_{100} of radiation and 1.0 and 0.5 of a maximally tolerated drug dose (MTD) established from drug toxicity testing. As seen in Table 12, a dose of 650 mg/kg was toxic in all cases. An oral dose of 325 mg/kg produced

Table 9

SUMMARY OF PRELIMINARY RADIOPROTECTOR TESTING

APRIL 1983

Compound WR	Compound BN	Route of Admin.	Prelim. Tox. MTD (mg/kg)[a]	Radioprotection: Maximum Survival %	Radioprotection: At Drug Dose (mg/kg)	Radioprotection: Highest Dose Tested (mg/kg)	Radioprotection: Time Min.	Report No. Tox.	Report No. Radiat.	Rec[b]
638	BJ 76356	ip	595					2		X
		po	595					2		
1065	BJ 05030	ip	300	100	150	300	30	10	11	X
		po	300	100	300	300	30	10	11	
2721	BK 02762	ip	432	100	500	500	30	1,2	c	X
		po	720	100	325	325	30	1,2	9	
3689	BJ 78538	ip	893	100	800	800	30	1,2	5	X
		po	1488	90	500	1000	30	1,2	5	
44923	BJ 40025	ip	298					2		X
		po	826					2		
77913	BJ 78529	ip	>1734	90	1500	1500	30	1,2	9	X
		po	>2600	100	2000	2000	30	1,2	9	
91283	BK 39456	ip	300	70	200	400	15	10	10	X
135023	BK 24080	ip	300	0	-	500	30	9	9	
151327	BJ 40016	ip	472					2		X
		po	1312					2		

Table 9
(contd)

Compound WR	Compound BN	Route of Admin.	Prelim. Tox. MTD (mg/kg)[a]	Radioprotection: Maximum Survival %	Radioprotection: At Drug Dose (mg/kg)	Radioprotection: Highest Dose Tested (mg/kg)	Radioprotection: Time Min.	Report No.: Tox.	Report No.: Radiat.	Rec[b]
168643	BJ 44774	ip	484	50	300	600	15	1,2	5	X
		po	806	90	400	800	15	1,2	5	
169402	BK 46942	ip	<37.5	0	-	32	30	10	11	
176542	BJ 44569	ip	174	90	100	200	30	2	9	X
		po	806	30	68.5	550	30	2	9	
246536	BJ 39684	ip		80	65	522	15		5	X
246537	BJ 39693	ip		10	243	486	15		5	
247119	BJ 91531	ip	150	0	-	200	30	10	10	
247231	BJ 46778	ip	(>882)[d]	0	-	882	15		5	
248346	BJ 58509	ip		10	600	600	15		5	
248778	BJ 63377	ip	(~600)[d]	20	300	600	15		8	
248833	BJ 63993	ip	(>679)[d]	60	201	679	15		5	
248836	BJ 64007	ip		50	134	134	15		5	X
		ip		90	134	134	15		8	
248872	BJ 68461	ip	(>1014)[d]	0	-	1014	15		5	
249262	BJ 78618	ip	150	70	150	360	30	10	10	X

Table 9

(contd)

Compound WR	Compound BN	Route of Admin.	Prelim. Tox. MTD (mg/kg)[a]	Radioprotection: Maximum Survival %	Radioprotection: At Drug Dose (mg/kg)	Radioprotection: Highest Dose Tested (mg/kg)	Radioprotection: Time Min.	Report No.: Tox.	Report No.: Radiat.	Rec[b]
249319	BJ 82907	ip		10	37.5	600	30		5	
249597	BJ 90954	ip	(~300)[d]	50	300	600	15		5	
249598	BJ 90927	ip	(>1014)[d]	0	-	1014	15		5	
249599	BJ 90945	ip	(>600)[d]	100	300	600	15		5	X
		ip	(~400)[d]	40	200	800	15		8	
249600	BJ 90936	ip		70	300	600	15		5	X
		po		50	600	600	30		8	
249705	BK 01818	ip	68.2	0	-	68.2	15	5	5	
249706	BK 01827	ip	45.8	100	11.4	91.5	15		5	X
		ip		10	91.5	91.5	15		8	
249707	BK 01809	ip	186	0	-	186	15	5	5	
249708	BK 01836	ip		100	588	588	30		5	X
249716	BK 02164	ip		0	-	600	15		8	
249914	BK 12919	ip	<37.5	70	15	30	30	6	6	X
249915	BK 12900	ip	<37.5	20	7.5	30	30	6	6	
249939	BK 13194	ip	9.38	80	8	16	30	7	7	X

Table 9

(contd)

Compound WR	Compound BN	Route of Admin.	Prelim. Tox. MTD (mg/kg)[a]	Radioprotection: Maximum Survival %	Radioprotection: At Drug Dose (mg/kg)	Radioprotection: Highest Dose Tested (mg/kg)	Radioprotection: Time Min.	Report No. Tox.	Report No. Radiat.	Rec[b]
250021	BK 16211	ip	<75	90	15	60	30	7	7	X
250022	BK 16200	ip	<75	60	30	60	30	7	7	
250023	BK 16202	ip	9.38	50	16	16	30	7	7	
250083	BK 20653	ip	18.8	80	9	36	30	8	8	X
250084	BK 20662	ip	18.8	80	9	36	30	8	8	X
250121	BK 22200	po	(>1000)[d]	0	-	1000 (+48 hr)	30		9	
250281	BK 38182	ip	60	60	30	60	30	10	10	
250282	BK 38173	ip	51	70	60	60	30	10	10	X
250288	BK 39330	ip	150	40	300	300	30	10	10	
250292	BK 39465	ip	>600	0	-	1000	15	10	10	
250293	BK 39474	ip	75	70	75	150	15	10	10	X
250345	BK 45605	ip	>600	80	600	1200	15	10	10	X
250346	BK 45614	ip	75	20	75	150	15	10	10	
250392	BK 46871	ip	300	0	-	400	30	10	11	

Table 9

(contd)

Compound WR	Compound BN	Route of Admin.	Prelim. Tox. MTD (mg/kg)[a]	Radioprotection: Maximum Survival %	Radioprotection: At Drug Dose (mg/kg)	Radioprotection: Highest Dose Tested (mg/kg)	Radioprotection: Time Min.	Report No. Tox.	Report No. Radiat.	Rec[b]
250393	BK 46899	ip	<37.5	60	20	20	30	10		
250394	BK 46906	ip	75	60	80	80	30	10		
250397	BK 46933	ip	600	100	1200	1200	30	10		X
250455	BK 48606	ip	37.5	0	-	60	15	10		
250456	BK 48615	ip	>600	0	-	1200	30	10		

[a]All doses corrected for salt and/or water content. Maximally tolerated dose is the drug dose producing 10% or less lethality in groups of treated mice.

[b]Recommended for further study.

[c]Positive control for radioprotector screening.

[d]Quantity of drug not sufficient for toxicity testing. MTD estimated from radioprotector screening data.

Table 10

SUMMARY OF STUDIES

DETAILED EVALUATION OF AGENTS OF INTEREST TO WRAIR

Agent:WR	BN	Route	Test Type
2721	BK 02762	po	Acute toxicity, new bottle
		ip	Radioprotection vs. drug dose
		po	Radioprotection vs. drug dose
		po	Duration of protection
		ip	Dose modification factor
168643	BJ 44774	ip	Dose modification factor
		po	Dose modification factor
		ip	Duration of protection
		po	Duration of protection
3689	BJ 78538	po	Dose modification factor
		ip	Duration of protection
		po	Duration of protection
1065	BJ 05030	ip	Toxicity
		po	Toxicity
		ip	Radioprotector screen
		po	Radioprotector screen
77913	BJ 78529	ip	Radioprotector screen
		po	Radioprotector screen
176542	BJ 44569	ip	Radioprotector screen
		po	Radioprotector screen

Table 11

RADIATION DOSE MODIFICATION - WR 2721 (BK 02762)

ADMINISTERED IP 30 MINUTES PRIOR TO IRRADIATION

Drug Dose (mg/kg)*	Radiation Dose (rads)	30-Day Survivors (%)
500	1050	100
	1150	90
	1322	90
	1521	100
	1749	80
	2011	60
	2313	20

*Corrected for salt and water content

Table 12

DURATION OF PROTECTION - WR 2721 (BK 02762) ADMINISTERED

ORALLY TO MICE PRIOR TO IRRADIATION WITH 1000 RADS

Drug Dose (mg/kg)*	Min Prior to Irradiation	Drug-Related Lethality	30-Day Survivors (%)
650	15	6/10	0
	30	8/10	10
	45	8/10	20
	60	10/10	0
	90	3/10	70
325	15	0/10	0
	30	0/10	100
	45	0/10	100
	60	0/10	90
	90	0/10	100
0	-	0/10	0

*Corrected for salt and water content

significant protection when administered up to 90 minutes prior to irradiation. This study will be extended to determine the time at which the degree of protection drops below 50%.

Oral and intraperitoneal activity of WR 2721 was determined in a drug dose vs. radioprotection study as described in Table 13. This study provided information about the degree of protection vs. the fraction of the MTD when the radiation dose and time of drug delivery before irradiation remain constant at 1000 rads and 30 minutes. The data also provided information which establishes a dose for 50% protection to be used in the time of maximum protection studies. This dose is approximately 65 mg/kg for intraperitoneally administered WR 2721 and 250 mg/kg for orally delivered compound.

Future plans for WR 2721 entail completion of the detailed evaluation of WR 2721 administered ip and po. This includes completion of duration of protection studies, establishing times of maximum protection, and determination of DMF values for protection against intestinal injury.

WR 168643. WR 168643 is an orally effective protectant that is also the parent compound for ongoing synthesis efforts. Experiments were performed to evaluate the radiation dose modification factor for this compound and the duration of protection as the first steps in the agent's detailed evaluation. These results are summarized in Table 14. A time course of protection study indicated that orally administered WR 168643 protected mice slightly more effectively than the drug given ip. Radioprotection by WR 168643 diminished rapidly with time after drug administration by either route. An interval of 15 minutes between drug and irradiation was chosen for future studies since protection at 5 minutes is most likely a pharmacologic phenomenon rather than true protection. DMF values determined for WR 168643 were similar for drug administered ip and orally. A DMF of 1.1 was found for 300 and 600 mg/kg of drug given ip. DMFs of 1.1 and 1.3 were found for 300 and 600 mg/kg of drug given orally. These values are based on an LD_{50} of 907 rads for unprotected mice.

Table 13

RADIOPROTECTION: WR 2721 (BK 02762) PLUS RADIATION

AT 1000 RADS 30 MINUTES AFTER INJECTION

Route	Dose (mg/kg)*	Drug Related Lethality	30-Day Survivors (%)
ip	500	0/10	100
	300	0/10	100
	180	0/10	90
	108	0/10	90
	64.8	0/10	50
	38.9	0/10	0
po	500	1/10	80
	300	0/10	90
	180	0/10	10
	108	0/10	0
	64.8	0/10	0
	38.9	0/10	0

*Corrected for salt and H_2O content

Table 14

SUMMARY OF DETAILED EVALUATION OF WR 168643 (BJ 44774)

Duration of Protection at 1000 Rads: 30-Day Survivors (%)

Dose (mg/kg)[a]	Route of Admin.	Minutes Prior to Irradiation 5	15	30	45
300	ip	100	40	10	25
600	ip	80	40	50	50
300	po	100	70	60	20
600	po	100	90	60	30

Radiation Protection for Drug Delivered 15 Minutes Prior to Irradiation: 30-Day Survivors (%)

Dose (mg/kg)[a]	Route of Admin.	Radiation Dose (rads) 756	870	1000	1150	1322	1521	1749
300[b]	ip	100	90	50	0	0	-	-
600[c]	ip	80	100	80	0	0	0	-
300	po	90	90	60	10	0	-	-
600[b]	po	-	100	100	40	0	0	0

Radiation Survival Parameters for Drug Delivered 15 Minutes Prior to 1000 Rads

Dose (mg/kg)[a]	Route of Admin.	$LD_{50\|30}$ (95% CL) Rads	Probit Slope	DMF
300	ip	1000 (954-1048)	40.4	1.1
600	ip	1028 (982-1077)	22.7	1.1
300	po	1000 (935-1068)	16.8	1.1
600	po	1134 (1083-1187)	41.6	1.3

[a] Corrected for salt and water content
[b] 1/10 drug related lethalities at 870 rads
[c] 1/10 drug related lethalities at >56 rads

WR 3689. This agent is an orally active compound also of interest to WRAIR. This phosphorothiate differs from WR 2721 only by the addition of a terminal methyl group. Duration of protection ip and po, as well as dose modification factor orally were evaluated. These results are summarized in Table 15. This compound is protective when administered ip 15-45 minutes prior to irradiation. Protection is delayed with oral administration with no protection evident at 15 minutes and 100% protection produced when drug is given 45 minutes prior to irradiation. An oral dose of 500 mg/kg protects 80%-100% of treated animals up to two hours post drug treatment. A radiation dose response study of mice pretreated with oral doses of 250 and 500 mg/kg showed LD_{50} values of 1165 rads and 1360 rads, respectively. This corresponds to DMF values of 1.3 and 1.5 based on an LD_{50} of 907 rads for nonprotected irradiated mice.

WR 1065, WR 77913, and WR 176542. These agents which are analogs of WR 2721 and are known radioprotectors were submitted for full radioprotectant characterization. As a first step in this process, the agents undergo routine preliminary screening to establish baseline parameters for further studies and to provide a data base with which to compare efficacy of newly synthesized radioprotectants.

The results of initial toxicity testing with WR 1065 are shown in Table 16. This agent is more toxic than WR 2721 and was tolerated at a dose of 300 mg/kg when given ip or orally. The radioprotectant activity of this agent and of WR 77913 and WR 176542 are shown in Table 17. Each agent was administered ip or orally 30 minutes prior to irradiation with 1000 rads. WR 1065 protected 100% of exposed mice at maximally tolerated drug doses given ip and orally. Drug toxicity was found at an ip dose of 300 mg/kg, a dose not lethal in initial toxicity testing. WR 77913 at the highest doses tested produced 90% and 100% protection when administered ip or orally. WR 176542 produced a maximum of 90% protection when given ip but only 30% when given orally.

Table 15

SUMMARY OF DETAILED EVALUATION OF WR 3689 (BJ 78538)

Duration of Protection at 1000 Rads: 30-Day Survivors (%)

Dose (mg/kg)[a]	Route of Admin.	Minutes Prior to Irradiation						
		15	30	45	60	90	120	
100	ip	90	40	70	0			
200	ip	100	70	80	0			
250	po	0	30	100	80			
500	po	0	70	100 (100)[b]	80 (100)	100	90	

Radiation Protection for Drug Delivered 45 Minutes Prior to Irradiation: 30-Day Survivors (%)

Dose (mg/kg)[a]	Route of Admin.	Radiation Dose (rads)					
		1150	1322	1521	1749	2011	2313
250	po	60	0	0	0	0	0
500	po	100	60	10	0	0	0

Radiation Survival Parameters for Drug Delivered Orally 45 Minutes Prior to 1000 Rads

Dose (mg/kg)[a]	$LD_{50/30}$ (95% CL) Rads	Probit Slope	$LD_{50/7}$ (95% CL) Rads	Probit Slope	$LD_{50/6}$ (95% CL) Rads	Probit Slope
250	1165 (1139-1193)	41.6	1654 (1578-1732)	36.2	1824 (1687-1972)	12.7
500	1360 (1324-1397)	28.8	1876 (1747-2013)	14.4	2040 (1915-2173)	16.8

[a] Corrected for salt and water content
[b] Numbers in parentheses are the results of follow-up testing

Table 16

ACUTE TOXICITY OF WR 1065 (BJ 05030)

Vehicle	Dose (mg/kg)*	Route of Admin.	Deaths	30-Day Survivors (%)
H_2O	600	ip	5/5	0
	300		0/5	100
	150		0/5	100
	1200	po	5/5	0
	600		4/5	20
	300		0/5	100

*Corrected for salt and H_2O content

Table 17

PRELIMINARY RADIOPROTECTION WITH SELECTED AGENTS TO MICE RECEIVING 1000 RADS

WR	BN	Route of Admin.	Time Before Rad (min)	Drug Dose (mg/kg)*	Drug Related Lethality	30-Day Survivors (%)
1065	BJ 05030	ip	30	300	6/10	40
				150	0/10	100
				75	0/10	50
		po	30	300	0/10	100
				150	0/10	90
				75	0/10	10
77913	BJ 78529	ip	30	1500	1/10	90
				750	0/10	90
				375	0/10	80
				187.5	0/10	40
		po	30	2000	0/10	100
				1000	0/10	0**
				500	0/10	60
				250	0/10	30
176542	BK 44569	ip	30	200	3/10	50
				100	0/10	90
				50	0/10	10
				25	0/10	0
		po	30	550	7/10	0
				275	0/10	20
				137.5	0/10	20
				68.5	0/10	30

*Corrected for salt and water content.

**5/10 mice dying prior to Day 8 suggest infection in this group. No other group in this experiment had Day 3-Day 8 mortality.

C. RADIATION RESPONSE OF C57BL/6 MICE

Results of the annual confirmation of the radiation response of C57BL/6 female mice are described in Table 18. Response parameters were calculated by the Cornfield-Mantel modification of Karber's method (Cancer Chemotherapy Reports 9:120-139, 1960). The $LD_{50/30}$ ± 95% CL was 907 rads (874-941) with a probit slope of 53. The $LD_{99/30}$ was calculated to be 1004 rads resulting in an estimated $LD_{100/30}$ of 1054 rads. These values are in relatively good agreement with our historical data.

Table 18

ANNUAL CONFIRMATION OF RADIATION RESPONSE

OF C57BL/6 FEMALE MICE

Radiation Dose (Rads)	30-Day Survivors (%)
613	100
693	100
783	100
885	70
1000	0
1130	0
1277	0
1443	0

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